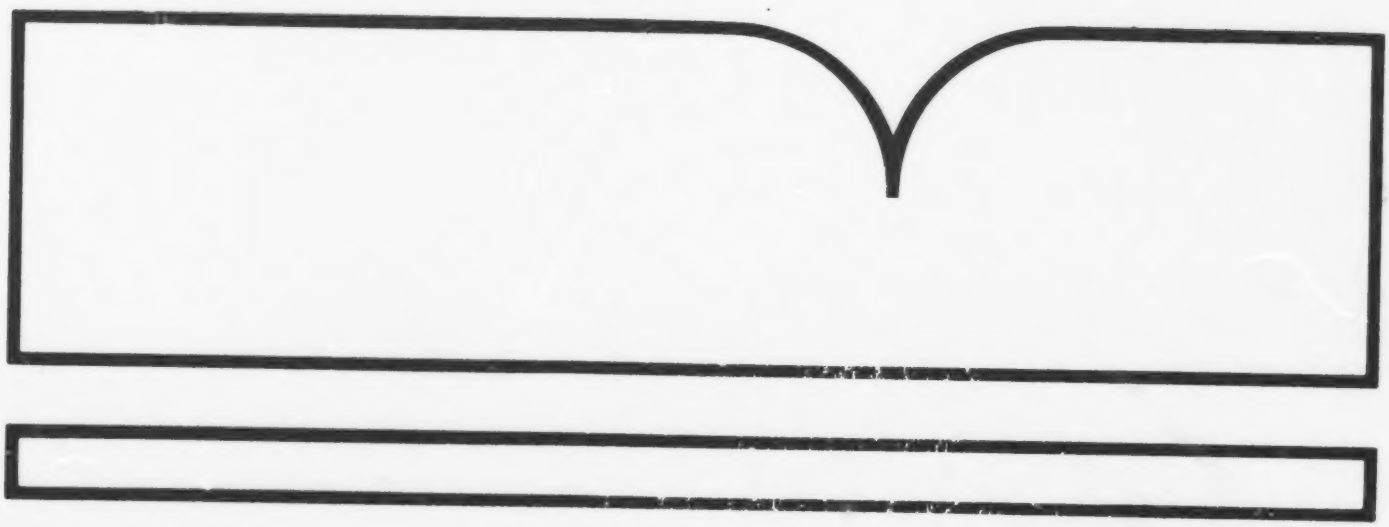
PB85-219699

Effect of Acute Maternal Toxicity on Fetal Development in the Mouse

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1985



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1. REPORT NO. EPA/600/J-85/060	2.
3. RI PB85-219699	
4. TITLE AND SUBTITLE The Effect of Acute Maternal Toxicity on Fetal Development in the Mouse (Journal Version)	5. REPORT DATE 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Robert J. Kavlock, Neil Chernoff, and Ellen H. Rogers	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Developmental Biology Division Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	10. PROGRAM ELEMENT NO. ACAE1A
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development US Environmental Protection Agency Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Teratogenesis, Carcinogenesis, and Mutagenesis 5(1): 3-13, Feb 1985

16. ABSTRACT

The effects of acute alterations in maternal health status upon fetal development were assessed following exposure of pregnant CD-1 mice on day 8 of gestation to one of ten chemicals at a dose calculated to be the maternal LD10 or LD40. The dams were killed on day 18 of gestation and the fetuses were examined by routine teratological techniques. The chemicals were cacodylic acid, caffeine, deltamethrin, dinoseb, ethylene bisisothiocyanate (EBIS), endrin, guthion, kepone, sodium salicylate, and toxaphene. Three (cacodylic acid, EBIS and kepone) produced dose related increases in the incidence of fully reabsorbed litters. Prenatal mortality in litters that contained live fetuses at term was elevated only for one chemical (cacodylic acid). Fetal weight was reduced in three instances (cacodylic acid, endrin, and guthion), while the incidence of terata was markedly elevated for two (cacoydlic acid and kepone). For two other chemicals (endrine and sodium salicylate), a low incidence was found of defects that were similar to defects induced by those chemicals in other species. These effects appear to be chemospecific in nature and not the result of some indirect maternal action. Thus, maternal health status, as measured by the incidence of lethality in the treated groups and by the magnitude of maternal weight gain in surviving females, presents no simple explanation for many manifestations of fetal toxicity. However, for seven chemicals (excluding deltamethrin, EBIS, and kepone) an increased incidence of supernumerary ribs was observed. For three of these seven chemicals (ceffeine, dinoseb, and toxaphene), supernumerary ribs was the only observed fetal effect. There was a significant linear inverse relationship between maternal weight gain during gestation and the incidence of extra ribs in the treated groups compared to their respective controls.

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a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	12
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE $7.00

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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JOURNAL ARTICLE

Teratogenesis, Carcinogenesis, and Mutagenesis 5:3–13 (1985)

The Effect of Acute Maternal Toxicity on Fetal Development in the Mouse

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The effects of acute alterations in maternal health status upon fetal development were assessed following exposure of pregnant CD-1 mice on day 8 of gestation to one of ten chemicals at doses calculated to exert either a low or a moderate degree of maternal lethality. The dams were killed on day 18 of gestation, and the fetuses were examined by routine teratological techniques. The chemicals were cacodylic acid, caffeine, deltamethrin, dinoseb, ethylene bisisothiocyanate sulfide (EBIS), endrin, guthion, kepone, sodium salicylate, and toxaphene. Three (cacodylic acid, EBIS, and kepone) produced dose-related increases in the incidence of dams with completely resorbed litters. Prenatal mortality in litters that contained live fetuses at term was elevated only for one chemical (cacodylic acid). Fetal weight was reduced in three instances (cacodylic acid, endrin, and guthion), while the incidence of terata was markedly elevated for two (cacodylic acid and kepone). For two other chemicals (endrin and sodium salicylate), a low incidence was found of defects that were similar to defects induced by those chemicals in other species. These effects appear to be chemospecific in nature and not the result of some indirect maternal action. Thus, maternal health status, as measured by the incidence of lethality in the treated groups and by the magnitude of maternal weight gain in surviving females, presents no simple explanation for many manifestations of fetal toxicity. However, for seven chemicals (excluding deltamethrin, EBIS, and kepone) an increased incidence of supernumerary ribs was observed. For three of these seven chemicals (caffeine, dinoseb, and toxaphene), supernumerary ribs was the only observed fetal effect. There was a significant linear inverse relationship between maternal weight gain during gestation and the incidence of extra ribs in the treated groups compared to their respective controls. Under the experimental conditions of this study, it appears that the incidence of supernumerary ribs increased in response to a nonspecific maternal toxicity.

Key words: maternal toxicity, supernumerary ribs, teratology, fetal toxicity, pesticides, mouse

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This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

INTRODUCTION

Regulatory guidelines for the determination of the teratogenic potential of chemicals frequently call for dose-response studies in pregnant rodents, with the high dose being of sufficient magnitude so as to induce maternal toxicity (such as slight weight loss but not more than 10% maternal deaths) and the lower doses some descending multiple of that dose [1]. The rationale behind the selection of a maternal toxic dose level is to maximize the ability to detect lesions in the offspring by administering the highest possible dosage to the dam. Ideally, effects observed in the offspring at the maternal toxic dose level are used as a beacon to focus attention on similar effects at lower dosages. Thus, if a statisically significant incidence of a particular defect is noted in the high-dose group, the biological significance of a lower and perhaps statistically non-significant incidence at lower dose levels is magnified. Difficulties arise, however, in interpreting effects that are observed only at maternally toxic dose levels. Do they represent specific, direct effects of chemical exposure on the conceptus or are they an indirect effect of non-specific alterations in maternal homeostasis? Some authors [2,3] have argued the latter point, but it is apparent from the literature that manifestation of maternal toxicity can occur without a noticeable impact on fetal development. For example, in a study of the teratogenic potential of the insecticide endrin, we [4] observed no effect on fetal development in rats despite a maternal weight gain in females from the high-dose group that was only 13% of that in control females.

Recently, Khera reveiwed over 200 teratology studies in mice with the goal of examining the relationship between maternal-fetotoxicity and teratogenicity [5]. Of 24 chemicals producing maternal toxicity with associated increased embryonic resorptions or reductions in fetal weight, three produced no fetal malformations, five produced a diverse pattern of anomalies, while the remainder produced a pattern of defects including exencephaly; hemivertebrae; fused vertebral arches or centra; and fused, missing, or supernumerary ribs. He concluded that the possibility could not be dismissed that maternal-fetotoxicity or fetotoxicity alone may have a contributory role in the malformations observed in the latter group. However, he did not report in that presentation on all situations involving maternal toxicity, only those with concurrent signs of fetotoxicity.

In this study we examined the possible relationships between maternal toxicity (in the form of lethality and reduced weight gain) and fetal outcome. A total of ten chemicals were administered to CD-1 mice on day 8 of gestation at dose levels predicted to induce either a low or a moderate degree of maternal lethality. We were interested in determining whether any particular fetal effects would be associated with the non-specific maternal effects of lethality and reduced weight gain. Day 8 of gestation was chosen as the day of treatment in the expectation that any adverse effects on maternal health that would induce a fetal effect might manifest itself in a syndrome of malformations of the central system which is rapidly developing at this time. A syndrome of defects occurring after exposure to such a wide spectrum of chemicals as used here would provide evidence that it was not the result of a specific effect of the chemical on the embryo, but rather that it represents a non-specific embryonic response to overall toxicity. If such effects could be observed following an acute exposure regimen such as this, then other studies could be conducted to determine whether alterations in maternal health status that occur at other critical periods or throughout the period of organogenesis would also result in non-specific fetal effects.

METHODS

Animals

Random-bred CD-1 mice, weighing between 29 and 33 g, were obtained from Charles River Breeding Laboratory. Toxicity studies were conducted on nulliparous mice, while primiparous animals received in our laboratory on day 2 of pregnancy (the morning of confirmation of the presence of a vaginal plug being day 1) were used in the teratology studies. All animals were housed on pine shavings in groups of five in plastic cages and maintained on a 12:12 photoperiod with food (Wayne Lab Blox) and water available ad libitum. Animals were received in weekly blocks during the course of the experiment, with each block representing the complete toxicity or teratology study for a particular compound. Each block contained an appropriate concurrent control. Within a block, animals were randomly assigned to one of the available treatment groups.

Compounds

The selection of chemicals for testing generally reflected our interest in the effects of pesticides on prenatal development. Cacodylic acid, an organic arsenical herbicide, (Lot 919-38-140, assay purity 99.5%) was obtained from the Ansul Company (Weslaco, TX); caffeine (Lot 107C-0045) from Sigma Chemical Co. (St. Louis, MO); deltamethrin, a synthetic pyrethroid insecticide, (technical grade) from Roussel Uclaf (Romaineville, France); dinoseb, a dinitrophenol pesticide, (technical grade, Lot AGR 133942, purity 97%) from Dow Chemical Co. (Midland, MI); EBIS, a breakdown product of the dithiocarbamate fungicides, (ethylenebisisocyanate sulfide, purity 98%) from Trans World Chemicals (Silver Spring, MD); endrin, a cyclodiene insecticide (Technical Reference Standard, Lot 3112-3) from Velsicol Chemical Co. (Chicago, IL); guthion, a chlorinesterase-inhibiting insecticide (Technical grade, Control No. 411-0229) from Chemagro, Inc., Kansas City, MO; kepone, a chlorinated hydrocarbon insecticide (recrystallized twice from toluene, Lot 6-6.11.74) from Allied Chemical Co., Morristown, NJ; sodium salicylate (Lot 764982) from Fisher Scientific; and toxaphene, a chlorinated camphor insecticide (Lot No. X16189-49, chlorine content 68.9%), from Hercules, Inc., Wilmington, DE. Compounds were administered as solutions in corn oil with the exception of cacodylic acid, caffeine, and sodium salicylate, for which distilled water was used as the vehicle. Intubation volumes were 0.5 ml/mouse.

Toxicity Studies

Toxicity studies for each compound were performed in nulliparous mice. Initial doses selected were based either on the acute LD50 values obtained from the literature or from extrapolation of sub-chronic toxicity studies done in our laboratory for routine teratology bioassays. Five groups of ten animals each were assigned to dose groups bracketing the anticipated LD50 value. Animals were observed for death and other signs of toxicity for 10 days following dosing. Probit analysis was used to calculate a dose that would induce a low degree of maternal lethality (the predicted LD10) and one that would induce a moderate degree of maternal lethality (the predicted LD40) for use in the teratology studies.

Teratology Studies

On day 8 of gestation, primiparous females were weighed and dosed with either the vehicle (20 animals) or a dose that induced a low degree of maternal lethality (20 animals) or a moderate degree of lethality (40 animals). Actual dosages used in these

studies can be found in Table 1. Animals were killed by decapitation on day 18 of gestation. The uterus was then removed and weighed. Maternal weight gain was calculated as the difference between the overall weight gain during pregnancy and the gravid uterus weight. The fetuses were removed from the uterus, blotted and weighed as a group, examined for gross malformations, and divided equally for fixation in either a solution of 5% formaldehyde, 5% glacial acetic acid, and 70% alcohol or in 70% alcohol alone. Using procedures described previously [4] the former were necropsied, while the latter were stained with alizarin red S and examined for skeletal abnormalities and maturity. Ammonium sulfide staining of the uteri from apparently non-pregnant females was not done, and it is possible that resorptions of entire litters that occurred shortly after treatment may have been overlooked. However, this

TABLE I. Maternal effects of exposure to ten chemicals at low and moderately maternally toxic dose levels

Compound	Dose (mg/kg)	No. treated	No. (%) maternal deaths	No. (%) whole litter resorptions[a]	No. (%) viable litters[a]	Initial maternal weight (g)[b]	Maternal weight gain (g)[b]
Cacodylic acid	0	33	0 (0)	0 (0)	30 (91)	32.0 ± 0.2[c]	3.7 ± 0.3
	1,600	19	5 (26)	3 (20)	7 (50)	31.4 ± 0.7	1.9 ± 0.4[d]
	2,400	40	4 (10)	12 (33)	14 (39)	32.4 ± 0.5	2.7 ± 0.3[d]
Caffeine	0	15	0 (0)	0 (0)	10 (67)	31.0 ± 0.8	3.3 ± 0.4
	350	20	5 (25)	0 (0)	8 (53)	32.9 ± 0.7	2.0 ± 0.6
	440	40	19 (47)	1 (5)	12 (57)	31.7 ± 0.6	2.4 ± 0.4
Deltamethrin	0	15	0 (0)	0 (0)	12 (80)	33.2 ± 1.1	3.7 ± 0.5
	30	20	1 (5)	1 (5)	13 (68)	31.3 ± 0.7	4.2 ± 0.3
	38	40	14 (35)	0 (0)	18 (69)	33.4 ± 0.7	3.0 ± 0.4
Dinoseb	0	15	0 (0)	0 (0)	12 (80)	28.0 ± 0.3	4.3 ± 0.8
	26	20	0 (0)	0 (0)	17 (85)	27.7 ± 0.4	4.6 ± 0.3
	33	40	2 (5)	1 (3)	20 (53)	27.6 ± 0.3	3.6 ± 0.5
EBIS	0	40	0 (0)	0 (0)	12 (80)	28.6 ± 0.4	4.4 ± 0.3
	400	20	0 (0)	4 (20)	4 (20)	29.1 ± 0.7	4.2 ± 0.4
	550	40	5 (13)	11 (31)	1 (3)	31.2	3.9
Endrin	0	40	0 (0)	2 (5)	28 (70)	32.2 ± 0.3	3.8 ± 0.3
	7	21	3 (14)	0 (0)	14 (78)	31.8 ± 0.4	2.7 ± 0.5[d]
	9	40	19 (47)	0 (0)	16 (76)	31.9 ± 0.5	3.0 ± 0.3
Guthion	0	15	0 (0)	0 (0)	13 (87)	32.1 ± 0.4	3.6 ± 0.3
	16	20	1 (5)	0 (0)	18 (95)	32.3 ± 0.5	3.4 ± 0.3
	20	40	21 (53)	0 (0)	13 (68)	33.1 ± 0.7	2.9 ± 0.4
Kepone	0	15	0 (0)	0 (0)	14 (93)	29.4 ± 0.6	3.8 ± 0.4
	110	20	5 (25)	1 (7)	12 (80)	29.4 ± 0.6	5.4 ± 0.3[d]
	125	40	17 (43)	7 (30)	9 (39)	30.4 ± 0.9	5.2 ± 0.4[d]
Na salicylate	0	15	0 (0)	0 (0)	13 (87)	29.9 ± 0.7	4.3 ± 0.3
	2,000	19	2 (11)	0 (0)	12 (71)	31.8 ± 0.8	2.5 ± 0.5[d]
	2,600	37	9 (24)	1 (4)	22 (79)	30.6 ± 0.5	3.1 ± 0.3[d]
Toxaphene	0	14	0 (0)	0 (0)	14 (100)	30.1 ± 0.8	5.2 ± 0.7
	100	20	1 (5)	0 (0)	17 (90)	32.1 ± 0.7	2.5 ± 0.3[d]
	120	40	7 (17)	0 (0)	27 (82)	32.8 ± 0.4	2.6 ± 0.2[d]

[a]Expressed as a percentage of those females surviving to term.
[b]Includes only those females that had live fetuses at term.
[c]Data expressed as mean ± standard error of the mean.
[d]Significantly different from concurrent control value.

particular response would manifest itself as an increase in the percent of treated females which survived to the scheduled sacrifice that were "non-pregnant."

Statistics

The litter was regarded as the fundamental unit of comparison in statistical procedures. For continuous variables, analysis of variance procedures available in the Statistical Analysis System [6] were used to detect overall treatment effects; post hoc t-tests were used to compare individual treatment groups when there was a significant F value in the analysis of variance (ANOVA).

RESULTS

Maternal Toxicity

The maternal effects of exposure to ten chemicals on day 8 of gestation at low and moderate levels of maternal lethality are presented in Table 1. Maternal lethality was observed for all chemicals, and with the exception of cacodylic acid, the incidences of mortality were dose-related. The magnitude of the mortality was generally in line with the desired levels of 10% and 40%. Differences between the predicted and observed lethalities probably reflect inherent difficulties in quantitative repetition of acute toxicity studies [7], as well as differences in the reproductive status of the animals [8] used in the dose-finding studies (nulliparous) and those in the teratogenicity studies (primiparous).

For three chemicals (cacocylic acid, EBIS, and kepone) a high incidence of whole litter resorptions was observed, but this was not correlated with the incidence of maternal lethality (e.g., the high-dose endrin and kepone groups both exhibited nearly 50% maternal mortality, and yet only kepone induced whole litter resorptions, while EBIS induced a low degree of maternal lethality and yet a high incidence of whole litter resorptions). Maternal weight gain during pregnancy was significantly reduced after exposure to four chemicals (cacodylic acid, endrin, sodium salicylate, and toxaphene) and increased after one (kepone). As expected, owing to the initiation of treatment after implantation, the total number of implantations sites were not affected by any treatment (data not shown). It should be noted that while these maternal effects were largely dose related in occurrence, there are instances in which the low dose is affected while the high dose is not (e.g., maternal weight gain in the case of endrin). Such U-shaped dose response curves may be biologically real, as the high degree of maternal mortality that occurred in the high-dose group may have eliminated more sensitive members of the population from examination at term. A similar inverse correlation between the induction of terata and embryonic death is well documented with increasing doses of many teratogens [9].

Fetal Toxicity

Fetal effects of exposures to the ten chemicals at maternally toxic dose levels are presented in Table 2. For only one treatment (cacodylic acid) did the incidence of prenatal mortality increase in litters that contained live fetuses at term. The incidence of prenatal mortality was significantly lower in toxaphene-exposed litters than in the concurrent control litters. Fetal weight was reduced by three treatments (cacodylic acid, endrin, and guthion). Delays in ossificiation of the skeleton, as measured by the number of sternal and caudal ossification centers, were generally observed only in

TABLE II. Fetal effects of exposure to ten chemicals at low and moderately maternally toxic dose levels

Compound	Dose (mg/kg)	No. litters[a]	Percent prenatal mortality	Weight (g)	No. sternal ossifications	No. caudal ossifications	Percent enlarged cerebral ventricles	Percent enlarged renal pelvis
Cacodylic acid	0	30	6 ± 1[b]	1.18 ± 0.04	5.9 ± 0.1	5.7 ± 0.4	4 ± 2	3 ± 1
	1,600	7	31 ± 11[c]	0.89 ± 0.06[c]	5.4 ± 0.4[c]	3.9 ± 0.5[c]	39 ± 20[c]	8 ± 6
	2,400	14	30 ± 7[c]	0.97 ± 0.04[c]	5.8 ± 0.1	4.3 ± 0.2[c]	6 ± 4	24 ± 10[c]
Caffeine	0	10	7 ± 2	1.07 ± 0.02	5.9 ± 0.1	4.4 ± 0.2	3 ± 2	2 ± 2
	350	8	9 ± 3	1.02 ± 0.03	5.8 ± 0.1	4.1 ± 0.3	2 ± 2	2 ± 2
	440	11	13 ± 4	1.00 ± 0.04	5.8 ± 0.2	3.9 ± 0.3	2 ± 2	6 ± 3
Deltamethrin	0	11	10 ± 3	1.03 ± 0.03	5.9 ± 0.1	4.4 ± 0.3	3 ± 2	0 ± 0
	30	13	10 ± 3	0.98 ± 0.04	5.8 ± 0.1	4.1 ± 0.4	4 ± 3	2 ± 2
	38	17	4 ± 1	1.05 ± 0.03	5.8 ± 0.1	4.5 ± 0.3	2 ± 1	1 ± 1
Dinoseb	0	11	5 ± 2	1.00 ± 0.04	5.4 ± 0.5	3.9 ± 0.4	4 ± 3	6 ± 4
	26	17	6 ± 2	1.03 ± 0.01	6.0 ± 0.1	4.2 ± 0.1	4 ± 2	0 ± 0[c]
	33	20	4 ± 1	0.98 ± 0.02	6.0 ± 0.1	4.1 ± 0.1	0 ± 0	1 ± 1[c]
EBIS	0	12	3 ± 1	1.00 ± 0.03	5.8 ± 0.1	3.8 ± 0.2	11 ± 3	3 ± 2
	400	4	2 ± 2	0.99 ± 0.06	5.9 ± 0.1	4.1 ± 0.6	5 ± 5	0 ± 0
	550	1	23	1.01	6.0	4.6	0	0
Endrin	0	27	8 ± 1	1.25 ± 0.04	5.9 ± 0.0	6.3 ± 0.4	5 ± 2	4 ± 2
	7	14	3 ± 2	1.05 ± 0.06[c]	5.7 ± 0.1[c]	5.1 ± 0.7[c]	4 ± 2	13 ± 5
	9	16	4 ± 2	1.20 ± 0.05	6.0 ± 0.1	5.9 ± 0.5	8 ± 3	7 ± 3
Guthion	0	13	12 ± 4	1.11 ± 0.04	5.9 ± 0.1	4.9 ± 0.4		8 ± 8
	16	18	8 ± 2	1.05 ± 0.03	5.9 ± 0.1	4.5 ± 1.1	0 ± 0	0 ± 0
	20	13	9 ± 2	0.99 ± 0.03[c]	5.9 ± 0.1	4.2 ± 0.3	0 ± 0	3 ± 2
Kepone	0	14	8 ± 3	1.02 ± 0.02	6.0 ± 0.0	4.2 ± 0.3	10 ± 5	1 ± 1
	110	12	8 ± 2	1.06 ± 0.04	5.9 ± 0.1	4.0 ± 0.2	3 ± 3	5 ± 4
	125	9	10 ± 3	0.99 ± 0.03	5.8 ± 0.1[c]	3.6 ± 0.2[c]	20 ± 11	2 ± 2
Na salicylate	0	13	7 ± 2	1.14 ± 0.05	5.9 ± 0.0	5.0 ± 0.3	2 ± 2	0 ± 0
	2,000	12	14 ± 5	1.02 ± 0.05	5.4 ± 0.4[c]	4.0 ± 0.4	0 ± 0	3 ± 2
	2,600	22	7 ± 2	1.16 ± 0.03	5.9 ± 0.1	5.5 ± 0.3	0 ± 0	1 ± 1
Toxaphene	0	14	10 ± 3	1.11 ± 0.04	5.9 ± 0.0	4.9 ± 0.3	5 ± 3	4 ± 4
	100	17	3 ± 1[c]	1.08 ± 0.04	5.9 ± 0.1	5.0 ± 0.3	5 ± 2	3 ± 2
	120	28	4 ± 1[c]	1.06 ± 0.02	5.9 ± 0.1	4.8 ± 0.3	9 ± 3	1 ± 1

[a]Includes only litters with live fetuses at term.
[b]Data presented as mean ± standard error of the mean.
[c]Significantly different from concurrent control value, $P < 0.05$.

groups that were retarded in overall growth, although in two cases (endrin and kepone) deficits in skeletal development remained after removing the influence of fetal weight by covariance analysis (analysis not shown). Major delays in the development of the cerebral ventricles and the renal papilla resulted from exposure to cacodylic acid, while renal development in dinoseb treated fetuses was significantly advanced compared to concurrent controls.

The most consistent pattern noted for any variable in the entire data set was significant increases in the incidence of supernumerary lumbar ribs. These were observed for seven of the ten chemicals (Fig. 1), the exceptions being EBIS, deltamethrin, and kepone. The incidence of supernumerary ribs in control litters ranged from a low of 3% in the guthion block to a high of 24% in the EBIS block, with an

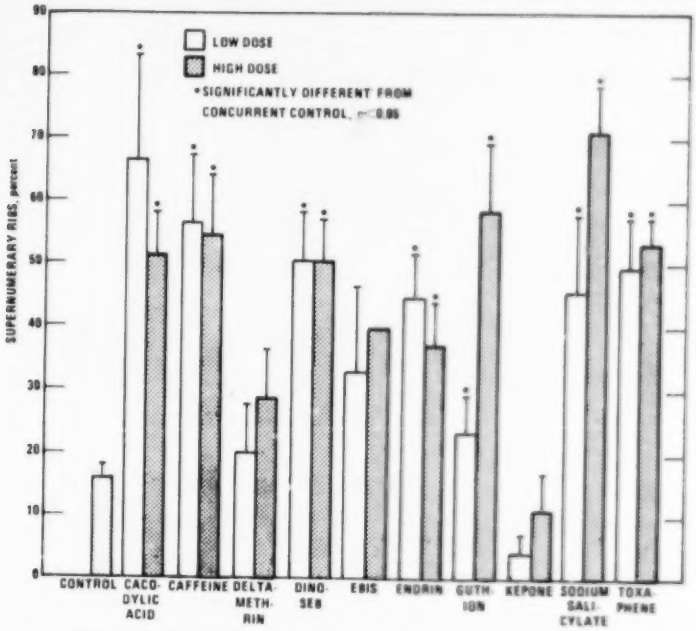

Fig. 1. The incidence of supernumerary ribs observed in fetuses exposed on day 8 of gestation to low or moderately maternally lethal dose levels.

overall average of 16%. In three instances (caffeine, dinoseb, and toxaphene), supernumerary ribs was the only significant finding in the fetuses. The percentage of fetuses displaying supernumerary ribs reached 72% in the high-dose salicylate group.

In support of the apparent correlation between alterations in maternal health status and the induction of supernumerary ribs, we analyzed the linear relationship between the change from concurrent control levels in the incidence of supernumerary ribs and the change in maternal weight gain relative to the same concurrent controls (Fig. 2). Maternal weight gain (as measured by the difference in maternal body weight at implantation and at term, less the weight of the gravid uterus) is a generally recognized quantitative index of maternal health status. The analysis depicted in Figure 2 indicates that there is a statistically ($p < 0.001$) linear inverse relationship between this measure of maternal health and the presence of supernumerary ribs in their offspring. As dams tended to gain less weight during gestation as a result of chemical exposure, their fetuses had a greater tendency to possess a lumbar rib. Other possible correlations, including the incidence of maternal mortality with extra ribs and with fetal weight and the change in maternal weight gain with fetal body weight alterations were also examined, although in only the latter case did the relationship approach statistical significance ($p < 0.07$).

Major malformations detected in fetuses either by visceral or skeletal examination are presented in Table 3. In control litters, a total of 12 out of 1,682 fetuses (0.7%) from 10 of 156 litters (6.3%) were malformed. The predominant lesions in control animals included encephaloceles, exencephalies, cleft palates, and hydronephrosis. Because of the low incidence of defects in control groups, we have chosen to

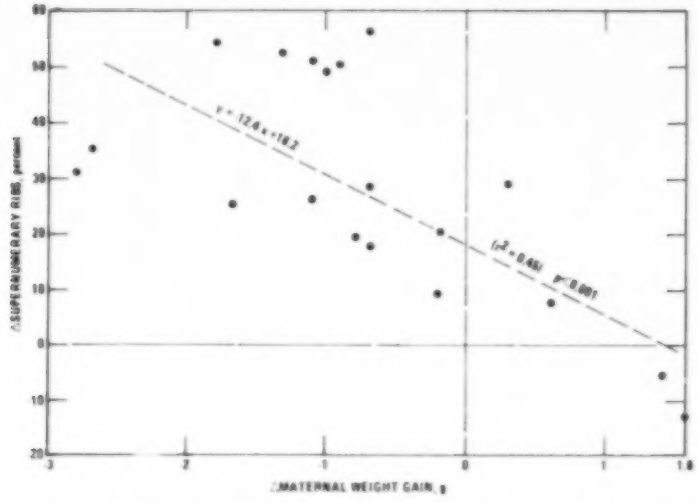

Fig. 2. Correlation between maternal weight gain and the incidence of supernumerary ribs. Each data point is derived from a single treatment group and are corrected for the values of the concurrent control group. Negative values indicate that treated groups were lower than their respective controls.

compare the response of treated groups to the entire control population rather than to their concurrent controls. The incidence of malformations in treated groups was elevated markedly for only two chemicals (cacodylic acid and kepone). In two other cases a low incidence was found of defects similar to defects induced by the same chemicals in other studies (fused ribs in endrin treated hamsters [10] and agnathia and fused vertebrae in salicylate treated rats [11]). Isolated cases of rather rare defects were found several times (phocomelia and testicular agenesis with decamethrin, an umbilical hernia with dinoseb, hemimelia with toxaphene). However, there clearly is no direct relationship between the induction of maternal toxicity and the production of major abnormalities. Patterns of malformations, when present, appeared to reflect more accurately the specific effect of the chemical on fetal development rather than a generalized toxicological response.

DISCUSSION

The relationship between acute alterations of maternal health status and fetal effects was examined for ten chemicals. Of all the fetal effects noted, only an increased incidence of supernumerary ribs was found in a majority of the treated groups. This effect was noted for seven of the chemicals, and appeared present in the EBIS group, although small sample sizes (four low-dose and one high-dose litter) prevented statistical significance in this instance. Examples of embryonic resorptions, of fetal growth retardations, and of teratogenesis were observed (Table 4), but these did not occur with sufficient regularity to be correlated with alterations in maternal health status. In particular, our initial hypothesis that the induction of maternal toxicity on day 8 of gestation would result in a syndrome of central nervous system defects owing to the vulnerability of the CNS during a period of rapid differentiation was not borne out. Rather, these effects on the developing conceptus appear to be chemical-specific in nature and not the result of some indirect action.

TABLE III. Malformations in fetuses from dams exposed to a low or moderately maternally toxic dose level of one of ten chemicals[1]

Compound	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Control[a]	0	1,682/156[b]	3/2[a]	2/2				2/1					1/1			3/3		1/1	1/6
Cacodylic acid	1,600	58/7	1/1	3/2	1/1	14/2		1/1				14/2	14/2	15/3		1/1			32/100
	2,400	123/14	9/4	4/2			1/1					4/3		11/6					19/64
Caffeine	350	79/8																	0/0
	440	109/11		1/1															1/9
Deltamethrin	30	142/13	1/1							1/1						1/1	1/1		3/31
	38	197/17	1/1														1/1		1/11
Dinoseb	26	181/17		1/1											1/1				1/12
	33	223/20	3/2			1/1													2/15
EBIS	400	43/4	2/1																5/25
	550	10/1																	0/0
Endrin	7	157/14		2/1															1/7
	9	184/16												3/1					2/6
Guthion	16	210/18	1/1																1/6
	20	145/13																	0/0
Kepone	110	126/12						1/1								2/1			2/17
	125	104/9	1/1									1/1	2/2	1/1					3/33
Na salicylate	2,000	114/12							2/1			2/1		3/1	2/1	1/1			7/17
	2,600	212/22	4/2									2/2		4/1					3/9
Toxaphene	100	214/17	2/1								1/1					1/1			2/12
	120	344/28										1/1	1/1			1/1			1/7

[1]A = Dose (mg/kg); B = No. fetuses/litters examined; C = Encephalocele; D = Exencephaly; E = Hydrocephaly; F = Miscellaneous cranial defects; G = Microphthalmia H = Cleft palate; I = Agnathia; J = Phocomelia; K = Hemimelia; L = Fused vertebral centrums; M = Fused vertebrae; N = Fused ribs; O = Umbilical hernia; P = Hydronephrosis; Q = Testicular agenesis; R = Short tail; S = Percent fetuses/litters with defects.

[a]Because of the low incidence of defects in the various concurrent control groups, the control data were pooled across all groups.

[b]Data presented as the No. fetuses affected/No. litters affected.

TABLE 4. Summary of observed effects in fetuses from dams surviving exposure to a low or moderately maternally toxic dose level of ten chemicals*

	Embryonic resorptions	Growth	Skeletal development	Supernumerary ribs	Terata
Cacodylic acid	↑	↓	↓	↑	↑
Caffeine	—	—	—	↑	—
Deltamethrin	—	—	—	—	—
Dinoseb	—	—	—	↑	—
EBIS	↑	—	—	—	—
Endrin	—	↓	↓	↑	↑
Guthion	—	↓	—	↑	—
Kepone	↑	—	↓	—	↑
Na salicylate	—	—	—	↑	↑
Toxaphene	↓	—	—	↑	—

*Vertical arrows indicate the presence and direction of statistically significant effects.

The biological significance of supernumerary ribs in rodent fetuses exposed to potential teratogens is far from understood. Yasuda and Maeda [12] suggested that the incidence of lumbar ribs in rats may serve as indicator of a chemical's teratogenic potential. Similarly in the rat, Kimmel and Wilson [13] concluded that extra ribs could be regarded as indicative of a teratogenic potential at some high dose level, providing one restricts their attention to supernumerary ribs that are at least half as long as the 13th rib. We examined the relative size distribution in the present study and found generally both classes of supernumerary ribs (rudimentary and extra) were elevated concurrently. Therefore, we did not distinguish between these categories in our presentation of the data. Russell [14] used the term "homeotic shifts" to describe evolutionarily based capabilities for alternative development in serial segments of the axial skeleton of the mouse and demonstrated using x-irradiation that such shifts have a high capability for revealing developmental disturbances. Interestingly, she found the critical period for production of posterior shifts of the thoraco-lumbar border occurred on 9½ of gestation (using day ½ to be the morning of the plug day). This corresponds quite well with our exposure period and also coincides with time of formation of the 23rd somite [15], indicating that the ultimate destiny of that member of the axial skeleton is determined at the time of its differentiation from the mesenchyme. More recently, Khera [16] reviewed some of the literature pertaining to "common fetal aberrations" and noted that there is a wide variation in the interpretation of the occurrence of supernumerary ribs, although he personally considers them of little or no significance. The present study indicates that they may be a response to a non-specific disruption in maternal health status.

In summary, under the exposure conditions and species used in this study, it is apparent that maternal health status, as measured by the incidence of lethality in treated groups and by the magnitude of maternal weight gain in surviving females, presents no simple explanation for many manifestations of fetal toxicity. There was no relationship between the incidence of maternal mortality in treated groups and any endpoints in the fetuses of surviving females in those groups. Only one effect that we observed, an elevation in the incidence of supernumerary ribs, was present in the majority of treated groups, and this was found to be inversely correlated with the magnitude of maternal weight gain. Further work is necessary to characterize more

precisely this correlation and to determine whether a cause-and-effect relationship exists. Because this study was limited to exposure on a specific day of gestation and the results may not be relevant to other exposure regimens, additional work is also necessary to extend these observations to other critical periods and to longer exposure periods.

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